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Prebiotics in food animals, a potential to reduce foodborne pathogens and disease[1]

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Abstract

Animals can be seriously impacted by bacterial pathogens that affect their growth efficiency and overall health, as well as food safety of animal derived products. Some pathogenic bacteria, such as Salmonella, can be a shared problem for both human and animal health, and can be found in many animal species. A fully-mature ecosystem (the intestinal tract) occupies all environmental niches and utilizes nearly all available nutrients, which tends to exclude pathogenic bacteria from the complex gastrointestinal microbial population. Utilization of this native or artificially-introduced microflora population to improve animal health and productivity has been termed a "probiotic", or competitive enhancement strategy. Advantages of harnessing the natural microbial ecosystem against the pathogens include ease of application and low economic and labor costs, and the use of a native microbial population to reduce transient pathogens is seen as a "natural" strategy. In this review, we will focus on the use of prebiotics and discuss the theory behind these compounds and their benefits, and challenges for future implementation in food animals.

Introduction

Animals can be seriously impacted by bacterial pathogens that affect both their growth efficiency and overall health, as well as food safety. Some pathogenic bacteria, such as *Salmonella*, can be a shared problem for both human and animal health, and can be found in many animal species. The intestinal microbial population of animals is very dense and highly diverse (Zoetendal *et al.,* 2006). More than 2000 bacterial species are known and populations $>10^{10}$ cells/g digesta are not uncommon (Hungate 1966). As the animal matures, there is a succession of species that colonize the gut and this population slowly increases in complexity, until a stable population becomes fully established (Lu *et al.,* 2003). A fully-mature ecosystem occupies all environmental niches and utilizes nearly all available nutrients, which tends to exclude pathogenic bacteria from the complex gastrointestinal microbial population.

Utilization of this native or artificially-introduced microflora population to improve animal health and productivity has been termed a "probiotic", or competitive enhancement strategy (Crittenden 1999; Fuller 1989). Advantages of using the natural microbial ecosystem against the pathogens include ease of application and low economic and labor costs, and the use of a native population to reduce transient pathogens is seen as a "natural" strategy. Collectively, competitive enhancement strategies include: probiotics, prebiotics and competitive exclusion cultures which, in some form, all utilize anti-pathogen activities of the

native (or introduced) microbial ecosystem via natural microbial competition. In this review, we will focus on the use of prebiotics and discuss the theory behind these compounds and their benefits, and challenges for future implementation in food animals.

Microbial ecology of the gut, competition and fitness

In many ways, the microecology of the intestinal tract is similar to the ecology of the macrobiological world; selective pressures that emphasize survival fitness occur in all environments, including microbial ones. The intestinal tract is very competitive, diverse and dense; comprised of over 2000 known species and a population in excess of 10^{10} cells/g digesta (Callaway *et al.,* 2010; Hungate 1966). The competition for nutrients and environmental niches is more intense than that found in the macrobiological jungle (Coleman *et al.,* 1996; Krause *et al.,* 1999). The scale of the intestinal environment means that dietary changes and other stressors result in environmental shifts that happen rapidly, necessitating an enhanced adaptability to opportunities and challenges by the bacterial population.

The synergistic relationship between the host animal and its gastrointestinal microbial ecosystem is critical to the health and well being of the animal and to efficient production (Jayne-Williams and Fuller 1971). The composition of the microbial population has been linked to the development of several conditions or diseases, at least in humans. It has been suggested that bacterial populations play a role in the development of autism (Bolte 1998; Finegold 2008; Finegold *et al.,* 2002; Murphy 2004). Other studies have linked human obesity with the population ratios of the phyla Firmicutes:Bacteroidetes (DiBaise *et al.,* 2008; Ley *et al.,* 2006; Turnbaugh *et al.,* 2009; Turnbaugh *et al.,* 2006). The benefits of the intestinal microbial population to food animals are due largely to the fermentation of dietary substrates to produce volatile fatty acids and B vitamins that are absorbed by the host animal (Branner and Roth-Maier 2006), but the native intestinal microbial population also stimulates the immune system (Koenen *et al.,* 2004; Schierack *et al.,* 2007; Walsh *et al.,* 2008) which can reduce colonization by pathogens and subsequent disease. This synergistic interaction between the host animal and its native microbial ecosystem that developed during millions of years of co-evolution, yet it has also been shaped by interactions between bacterial species within this consortium.

While the host/bacterial interaction is important, in this chapter, we will focus on harnessing the interbacterial competition and how that can affect animal health. The bacterial species best adapted to occupy a particular niche within the intestinal tract will become the most successful and will eventually come to dominate the niche. An established, mature, gastrointestinal microbial population fills all available environmental niches making an animal more resistant to colonization by opportunistic bacteria, especially pathogenic bacteria, which has been described as “bacterial antagonism”, “bacterial interference”, or “competitive exclusion” (Lloyd *et al.,* 1974; 1977; Nurmi and Rantala 1973). The modes of action that have been linked to gastrointestinal populations that eliminate pathogenic bacteria include: 1) direct and indirect competition for nutrients, 2) competition for physical attachment sites, 3) production of antimicrobial compounds (including Volatile Fatty Acids [VFA]), 4) enhancement of host immune system activity, and 5) a synergy between some of these activities. If bacteria (including pathogens) cannot grow at least as fast as the passage rate of their environment, then digesta flow will “wash out” the pathogen. Within the gut bacteria bind to the surface of the intestinal epithelium (Lloyd *et al.,* 1974; 1977) preventing opportunistic pathogens from attaching and thus obtaining a colonization foothold (Collins and Gibson 1999). Volatile fatty acids produced by normal microbial fermentation in the gut are toxic to some pathogenic bacteria, and may reduce the competitive fitness of these bacteria in the gut environment (Barnes *et al.,* 1979; Prohaszka and Baron 1983; Wolin 1969). Additionally, some commensal intestinal bacteria produce antimicrobial protein compounds, such as traditional

antibiotics and bacteriocins or colicins that can inhibit or eliminate species competing within the same niche (Jack *et al.,* 1995; Lee *et al.,* 2008; Xavier and Russell 2006).

Prebiotics, a targeted colonic food

While several "competitive enhancement" techniques have been used to modify the microbial population, the use of probiotic approaches offer intriguing promise at improving the microbial community's ability to prevent pathogen colonization or expansion. One of the most exciting of these techniques is the use of prebiotics. At their core, prebiotics simply are a specific limiting nutrient that is provided to the microbial population but is unavailable to (or unused by) the host (Walker and Duffy 1998). Thus prebiotics can provide a competitive advantage that can allow an existing (or added, in the case of synbiotics discussed below) commensal population to expand its niche to further exclude pathogenic bacteria (Crittenden 1999; Walker and Duffy 1998).

Prebiotics are organic compounds that are unavailable to, or indigestible by the host animal, but are available to a specific proportion of the microbial population and are often described as "functional foods" or "nutraceuticals" (Schrezenmeir and De Vrese 2001). Prebiotics have been most often used as dietary supplements in humans to promote intestinal health and well-being (Crittenden 1999). Some carbohydrates like oligosaccharides and other organic compounds such as inulin are not acidically, nor enzymatically degraded in the stomach or intestine and can reach the cecum and colon where they become "colonic food" for the microbial population in the small and large intestine (Houdijk *et al.,* 1998; Kontula 1999; Meyer 2008). Prebiotic inclusion in animal rations has been linked to an increase in diversity of the intestinal tract (Krause et al., 2010), and to a decrease in interanimal variability (Janczyk et al., 2010).

Some prebiotics provide a competitive advantage to specific members of the native microflora (e.g., *Bifidobacteria*, *Butyrivibrio, Lactobacillus*) (Kim et al., 2011; Nakashimada et al., 2011; Shen et al., 2010; Willard et al., 2000) that are known to act antagonistically against pathogens. Prebiotics may also provide some limiting nutrients directly to the intestinal mucosa, reduce colonic inflammation/colitis (Pouillart et al., 2010) and provides substrates for intestinal bacteria to ferment, which yields increased B vitamin production (Branner and Roth-Maier 2006; Collins and Gibson 1999; Leenen and Dieleman 2007). Researchers have found that prebiotic supplementation can also affect vilus height and crypt depth in the intestine(Awad et al., 2011). Galactooligosaccharides as prebiotics have been demonstrated to have anti-adhesive activity, reducing the adherence of a human enteropathogenic *E. coli* (EPEC) to the human cell lines HEp-2 and Caco-2 in a dose-dependent manner (Shoaf et al., 2006). Recent research has indicated that the use of inulin and oligofructans can directly modulate activity of the immune system (Bailey 2009; Meyer 2008; Seifert and Watz 2007), and can decrease allergic-asthma symptom in animals (Vos *et al.,* 2007). Further studies found that the use of prebiotics can increase the resistance of animals to infection and the incidence of atopic dermatitis (Meyer 2008). Other researchers have found that the inclusion of prebiotics can also affect animal behavior and well-being (Van Loo 2007), as well as their stress responses (Ghareeb et al., 2008).

Prebiotics in food animals

While much of the research into prebiotics has focused on the use in humans, prebiotics have been used in the animal feed industry to improve the health and well-being of poultry, swine, horses and dogs; however prebiotics remain relatively expensive for use in commercial animals (Mosenthin and Bauer 2000; Respondek *et al.,* 2008; Torres-Rodriguez *et al.,* 2007; Willard *et al.,* 2000). Prebiotics have also had somewhat limited application in

food animals commercially as pathogen-reduction strategies in food animals in part due to the availability of cheap antibiotics which can counteract the effectiveness of competitive enhancement strategies (Steer *et al.,* 2000). Given increasing fears over the dissemination of antimicrobial resistance, it is expected that prophylactic antibiotic usage in food animals will become more closely regulated and expensive, causing prebiotics to become more economically feasible and widely used in disease prevention.

Prebiotic studies in food animals have been characterized by inconsistency, primarily due to a lack of understanding of the microbial ecology of the gastrointestinal tract and conditions that promote the growth of pathogens and commensal organisms (Wiemann 2003). Some prebiotics were chosen that gave an advantage to bacterial species chosen for use in animals that were isolated from other sources and were thus not well suited for life in the anaerobic gut ecosystem, so the prebiotic treatment "failed" to prevent the pathogen colonization and was shelved. Additionally, variations between studies can further be attributed to antagonistic interactions between some bacterial species, as well as quality control issues. Mature animals contain a stable, relatively individualized intestinal microbial population, when prebiotics are applied to neonates with a (nearly) sterile intestinal tract results are more consistent. All of these factors have produced results that are in many cases, sadly unrepeatable. However, the advent of molecular methodologies has allowed more precise definitions of the effects of individual prebiotics, and a greater understanding of the "normal" gut flora and degree of individualization of the intestinal microbial ecosystem which can lead to the future development of highly tailored prebiotic products for use in specific animals or production environments (or for use in synbiotics).

Because chickens are typically grown in large groups (in some cases more than 100,000 birds in one house), one of the most important factors in determining which feedstuffs will be included in rations is cost due to the need to treat all birds simultaneously. In broilers, prebiotics have been generally included to improve body weight gain and feed conversion ratio, and they do have this effect in some studies (Corduk et al., 2008). Other research groups have found that mixed herbal pellets that contained prebiotic compounds, showed improved gain, feed conversion and mortality rates (Al-Kassi and Witwit 2010). Turkeys fed lactose as a prebiotic tended to be heavier when supplemented during the brooding and growout phase, both with and without co-addition of a lactobacillus culture (Torres-Rodriguez et al., 2007). Prebiotic supplementation with a maltooligosaccharide (MOS) however, has been shown to not impact any production parameters or IgG in broilers (Midilli et al., 2008).

Treatment of broilers with MOS and FOS resulted in a significant reduction in the proportion of B cells and in lymphocyte in cecal tonsils; FOS treatment significantly enhanced the IgM and IgG antibody titers in plasma (Janardhana et al., 2009). The inclusion of chicory in broiler diets increased the duodenal villus height, villus width and villus height to crypt depth ratios and decreased the villus height and crypt depth in the jejenum and ileum (Awad et al., 2011). Other studies have found that chicory feeding did not affect broiler performance (Da Silva et al., 2011). Others found that chicory feeding increased broiler resistance to stress, especially when coupled to probiotic feeding (Ghareeb et al., 2008).

Bifidobacteria are viewed as being very important to intestinal well being and animal health and are used as an indicator of prebiotic success, some of the the benefits of inclusion of xylooligosaccharides (XOS) and arabinoxylooligosachrides (AXOS) in broiler rations include increased Bifidobacterial populations (Courtin et al., 2008). Feeding of galactooligosaccharides (GOS) to broilers also increased Bifidobacterial counts in the intestinal tract (Jung et al., 2008). Feeding fructooligosaccharides (FOS) and MOS to broilers has been shown to decrease *Clostridia* and *E. coli* populations whilst increasing lactobacilli populations and diversity, as well as total bacterial populations (Kim et al., 2011). In this

same study, FOS and MOS feeding showed approximately the same productivity benefits as feeding the antibiotic avilamycin (Kim et al., 2011).

In swine, growth and animal health are critical to farm productivity, yet to date there has been little use of traditional oligosaccharide based prebiotics in swine. Fructooligosacchrides have been shown to have an effect on weight gain, but not diarrhea or feed efficiency (Budiño et al., 2010). Prebiotic (an alginate and inulin product) feeding to swine showed an increased homogeneity of DGGE profiles in the colon of swine compared to controls (Janczyk et al., 2010). However this effect was not observed in the small intestine, and production parameters were not reported (Janczyk et al., 2010). Lactulose feeding to piglets fed milk replacer reduced clostridial populations in the cecum, although the dose required often results in diarrhea (Kien et al., 2007); these results are probably most applicable to humans, especially in the realm of autism and the hypothesized link to clostridial involvement (Bolte 1998). When pigs were fed a prebiotic type fermentation endproduct (from *Saccharomyces cerevisiae*) increased fecal shedding of an experimentally infected *Salmonella*, and increased populations of *Bacteroides* and *Lactobacilli* in the intestinal tract but did not alter *Salmonella* populations or the duration of illness (Price et al., 2010). However there were greater compensatory gain in pigs fed this yeast prebiotic product. Dried skim milk is often used in swine rations for its purported prebiotic benefits, especially in regard to increasing *Lactobacillus* populations in the gut. Feeding of dried skim milk did not affect *Salmonella* or *Campylobacter* shedding in growing swine, but fewer recurring incidences of *Salmonella* shedding occurred in animals that maintained higher *Lactobacillus* populations which were correlated to dried skim (Wells et al., 2005). Thus in swine, some prebiotics have been shown to affect pathogenic bacterial pathogens of note to both human consumers and from an animal-health perspective.

Cattle, as ruminants, are very different than the monogastric food animals, the presence of a huge, dense pre-gastric microbial population in the rumen that is devoted to breaking down many of the common prebiotic compounds presents enormous challenges to the implementation of prebiotics in cattle. Another factor working against prebiotic usage in cattle is the large gastrointestinal tract volume, even excluding the rumen the GIT volume exceeds 100 liters (Russell 2002). This has limited the number of studies that have investigated the use of prebiotics in ruminants; however, enhancements in rumen-protective technologies may allow these compounds to be used in feedlot and dairy cattle. A prebiotic for use in cattle, Celmanax, acted as an anti-adhesive for Enterohemorrhagic *E. coli* (EHEC) colonization and a mycotoxin binder in in vitro studies (Baines et al., 2011). Although few prebiotics alone have been used, their promise for use as lower-intestinal tract adjuncts to probiotics (synbiotics) shows distinct promise (Yasuda et al., 2007). As the costs of inclusion of prebiotics as a rumen-protected part of a synbiotic directed anti-pathogen strategy may become economically feasible.

Synbiotics: a synergistic approach to harnessing the microbial ecology

Coupling the use of probiotics or competitive exclusion cultures and prebiotics is known as “synbiotics” (Branner and Roth-Maier 2006; Collins and Gibson 1999; Schrezenmeir and De Vrese 2001). Because these products can be tailored to support each other in a highly targeted fashion, this is often seen as the most likely approach to succeed in reducing pathogens in food animals (Vandeplas et al., 2010). In research studies, a synergistic effect in reduction of food-borne pathogenic bacterial populations in food animals prior to slaughter is often seen (Bomba *et al.,* 2002). By providing a specific limiting substrate/nutrient to a specific anti-pathogenic segment of the intestinal population, that

population can be retained to combat transient pathogens that can affect the host animal or human consumers.

Broiler studies that fed specific Lactobacilli and prebiotics to broilers have shown improvement in weight gain, feed conversion and health (Mátéová et al., 2008; Mokhtari et al., 2010). Feeding of a synbiotic to broiler chickens showed increased body weight gain and feed conversion, and the synbiotic effect was greater than either treatment alone (Falaki et al., 2010). Further synbiotic feeding has shown that broilers fed a chicory-based prebiotic along with a probiotic were less negatively affected by stress (Ghareeb et al., 2008), and other studies have indicated synbiotic usage reduces the effects of heat stress on broilers (Silva et al., 2010). In swine, administration of a mixture of a *Lactobacillus casei* culture and maltodextrins resulted in a reduction of approximately 1 $\log_{10}$ in adherence in gnotobiotic pigs and of about 2.5 $\log_{10}$s in "regular" pigs. The significance of these data is difficult to determine as the concentrations of ETEC in the intestine were much lower than those usually associated with natural or experimental ETEC infections. Another *L. plantarum* synbiotic containing maltodextrin and/or FOS reduced counts of diarrheagenic *E. coli* O8:K88 in the jejunum and colon of piglets, and was associated with increased acetate concentrations in the ileum and colon (Nemcova et al., 2007). A mixture of raw potato starch as a prebiotic with probiotic *E. coli* strains reduced the colonization of the swine with the diarrheagenic E. *coli* K88 in a challenge model (Krause et al., 2010). The use of the synbiotic treatment enhanced growth performance and decreased diarrhea and increased microbial diversity in early-weaned pigs (Krause et al., 2010). In cattle, a *Lactobacillus casei* culture coupled with dextran feeding resulted in a significant increase in Holstein cow milk production; including total milk, fat, protein and solids-non-fat production (Yasuda et al., 2007).

Conclusions

As our understanding of the complexities of the gastrointestinal microbial ecosystem has grown in recent years, so has interest in utilizing the natural power contained within this ecosystem as a tool in our arsenal to improve both animal and human health. The diversity of the microbial population of the intestinal tract and skin is a natural resource that can be harnessed, and stimulating the commensal (or beneficial members) of the native intestinal flora may make it more difficult for pathogenic bacteria to become established in food animals. Prebiotics offer an outstanding tool for utilizing the native microbial population against diseases of food animals. These products are indigestible by the host animal and provide food or a limiting nutrient to some or all members of the microbial population. Previous research with prebiotics in food animals has not always been successful because of a general lack of understanding of the microbial ecosystem and which ones were utilizing the prebiotic compounds. As molecular techniques have improved the depth and breadth of our understanding, we are now able to tailor prebiotic feeding to manipulate specific microbial populations. However, prebiotic prices currently remain high for use in commercial agriculture and thus are primarily associated with human foods and pet feeds. Yet as further research into prebiotics demonstrates their ability to prevent colonization of food animals with pathogens that affect human and animal health, the demand will increase, driving down costs and allowing widespread utilization in agricultural applications. Thus, by enhancing our knowledge of how the microbial population of the intestinal tract interacts with the animal and other members of the microbial ecosystem, we can further enhance growth efficiency, productivity, animal health and food safety.

References

1. AL-KASSI G.A.M. AND WITWIT N.M. 2010. A comparative study on diet supplementation with a mixture of herbal plants and dandelion as a source of prebiotics on the performance of broilers. Pak. J. Nutr. 9:67-71.
2. AWAD W.A., GHAREEB K. AND BÖHM J. 2011. Evaluation of the chicory inulin efficacy on ameliorating the intestinal morphology and modulating the intestinal electrophysiological properties in broiler chickens. J. Anim. Physiol. Anim. Nutr. 95:65-72.
3. BAILEY M. 2009. The mucosal immune system: Recent developments and future directions in the pig. Develop. Comp. Immunol. 33:375-383.
4. BAINES D., ERB S., LOWE R. et al., . 2011. A prebiotic, Celmanax, decreases *Escherichia coli* O157:H7 colonization of bovine cells and feed-associated cytotoxicity in vitro. BMC Res. Notes 4.
5. BARNES E.M., IMPEY C.S. AND STEVENS B.J.H. 1979. Factors affecting the incidence and anti-*Salmonella* activity of the anerobic cecal flora of the chick. J. Hyg. 82:263-283.
6. BOLTE E.R. 1998. Autism and *Clostridium tetani*. Med.l Hypoth. 51:133-144.
7. BOMBA A., NEMCOVÁ R., MUDRONOVÁ D. AND GUBA P. 2002. The possibilities of potentiating the efficacy of probiotics. Trends Food Sci. Technol. 13:121-126.
8. BRANNER G.R. AND ROTH-MAIER D.A. 2006. Influence of pre-, pro-, and synbiotics on the intestinal availability of different B-vitamins. Arch. Anim. Nutr. 60:191-204.
9. BUDIÑO F.E.L., JÚNIOR F.G.D.C. AND OTSUK I.P. 2010. Frutooligosaccharide addition in diets for weaned pigs: Performance, diarrhea incidence and metabolism. Rev. Brasil. Zootec. 39:2187-2193.
10. CALLAWAY T.R., DOWD S.E., EDRINGTON T.S. ET Al., . 2010. Evaluation of bacterial diversity in the rumen and feces of cattle fed different levels of dried distillers grains plus solubles using bacterial tag-encoded FLX amplicon pyrosequencing. J. Anim. Sci. 88:3977-3983.
11. COLEMAN M.E., DREESEN D.W. AND WIEGERT R.G. 1996. A simulation of microbial competition in the human colonic ecosystem. Appl. Environ. Microbiol. 62:3632-3639.
12. COLLINS D.M. AND GIBSON G.R. 1999. Probiotics, prebiotics, and synbiotics: approaches for modulating the microbial ecology of the gut. Amer. J. Clin. Nutr. 69:1052S-1057S.
13. CORDUK M., CEYLAN N., DEDE N. AND TEL O.Y. 2008. Effects of novel feed additives on performance, carcass traits and *E. coli*, aerobic bacteria and yeast counts in broilers. Archiv fuf Geflugelkunde 72:61-67.
14. COURTIN C.M., SWENNEN K., BROEKAERT W.F. et al., . 2008. Effects of dietary inclusion of xylooligosaccharides, arabinoxylooligosaccharides and soluble arabinoxylan on the microbial composition of caecal contents of chickens. J. Sci. Food Agric. 88:2517-2522.
15. CRITTENDEN R.G. 1999. Prebiotics. In *Probiotics: A critical review*. Tannock GW, ed. Horizon Scientific Press, Wymondham, UK. pp 141-156.
16. DA SILVA W.T.M., NUNES R.V., POZZA P.C., DOS SANTOS POZZA M.S., APPELT M.D. AND EYNG C. 2011. Evaluation of inulin and probiotic for broiler chickens. Acta Scient. Anim. Sci. 33:19-24.
17. DIBAISE J.K., ZHANG H., CROWELL M.D., KRAJMALNIK-BROWN R., DECKER G.A. AND RITTMANN B.E. 2008. Gut microbiota and its possible relationship with obesity. Mayo Clin. Proc. 83:460-469.
18. FALAKI M., SHARGH M.S., DASTAR B. AND ZREHDARAN S. 2010. Effects of different levels of probiotic and prebiotic on performance and carcass characteristics of broiler chickens. J. Anim. Vet. Adv. 9:2390-2395.
19. FINEGOLD S.M. 2008. Therapy and epidemiology of autism-clostridial spores as key elements. Med. Hypoth. 70:508-511.
20. FINEGOLD S.M., MOLITORIS D., SONG Y. et al., . 2002. Gastrointestinal microflora studies in late-onset autism. Clin. Infect. Dis. 35:S6-S16.
21. FULLER R. 1989. Probiotics in man and animals. J. Appl. Bacteriol. 66:365-378.
22. GHAREEB K., AWAD W.A., NITSCH S., ABDEL-RAHEEM S. AND BÖHM J. 2008. Effects of transportation on stress and fear responses of growing broilers supplemented with prebiotic or probiotic. Int. J. Poult. Sci. 7:678-685.
23. HOUDIJK J.G.M., BOSCH M.W., VERSTEGEN M.W.A. AND BERENPAS H.J. 1998. Effects of dietary oligosaccharides on the growth and faecal characteristics of young growing pigs. Anim. Feed Sci. Technol. 71:35-48.
24. HUNGATE R.E. 1966. *The Rumen and its Microbes*. Academic Press, New York, NY.
25. JACK R.W., TAGG J.R. AND RAY B. 1995. Bacteriocins of gram-positive bacteria. Microbiol. Rev. 59:171-200.
26. JANARDHANA V., BROADWAY M.M., BRUCE M.P. et al., . 2009. Prebiotics modulate immune responses in the gut-associated lymphoid tissue of chickens. J. Nutr. 139:1404-1409.
27. JANCZYK P., PIEPER R., SMIDT H. AND SOUFFRANT W.B. 2010. Effect of alginate and inulin on intestinal microbial ecology of weanling pigs reared under different husbandry conditions. FEMS Microbiol. Ecol. 72:132-142.

28. JAYNE-WILLIAMS D.J. AND FULLER R. 1971. The influence of the intestinal microflora on nutrition. In *Physiology and Biochemistry of the Domestic Food*. Bell DJ, Freeman BM, eds. Academic Press, London, UK. pp 74-92.
29. JUNG S.J., HOUDE R., BAURHOO B., ZHAO X. AND LEE B.H. 2008. Effects of galacto-oligosaccharides and a *Bifidobacteria lactis*-based probiotic strain on the growth performance and fecal microflora of broiler chickens. Poult. Sci. 87:1694-1699.
30. KIEN C.L., BLAUWIEKEL R., WILLIAMS C.H., BUNN J.Y. AND BUDDINGTON R.K. 2007. Lactulose feeding lowers cecal densities of clostridia in piglets. J. Parent. Enter. Nutr. 31:194-198.
31. KIM G.B., SEO Y.M., KIM C.H. AND PAIK I.K. 2011. Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. Poult. Sci. 90:75-82.
32. Koenen M.E., Kramer J., Van Der Hulst R., Heres L., Jeurissen S.H.M. and Boersma W.J.A. 2004. Immunomodulation by probiotic lactobacilli in layer- and meat-type chickens. Brit. Poult. Sci. 45:355-366.
33. KONTULA P. 1999. In vitro and in vivo characaterization of potential prebiotic lactic acid bacteria and prebiotic carbohydrates. Finn. J. Dairy Sci. 54:1-2.
34. KRAUSE D.O., BHANDARI S.K., HOUSE J.D. AND NYACHOTI C.M. 2010. Response of nursery pigs to a synbiotic preparation of starch and an anti-*Escherichia coli* K88 probiotic. Appl. Environ. Microbiol. 76:8192-8200.
35. KRAUSE D.O., MCSWEENEY C.S. AND FORSTER R.J. 1999. Molecular ecological methods to study fibrolytic ruminal bacteria: phylogeny, competition, and persistence. In *8th Int. Symp. Microbial Ecol.* Bell CR, Brylinsky M, Johnson-Green P, eds. (
36. LEE N.K., LEE J.Y., KWAK H.G. AND PAIK H.D. 2008. Perspectives for the industrial use of bacteriocin in dairy and meat industry. Korean Journal for Food Science of Animal Resources 28:1-8.
37. LEENEN C.H.M. AND DIELEMAN L.A. 2007. Inulin and oligofructose in chronic inflammatory bowel disease. J. Nutr. 137:2572S-2575S.
38. LEY R.E., TURNBAUGH P.J., KLEIN S. AND GORDON J.I. 2006. Human gut microbes associated with obesity. Nature 444:1022-1023.
39. LLOYD A.B., CUMMING R.B. AND KENT R.D. 1974. Competitive exclusion as exemplified by *Salmonella typhimurium*. In *Australasian Poult. Sci. Conv., World Poult. Sci. Assoc. Austral. Br.* pp 155.
40. LLOYD A.B., CUMMING R.B. AND KENT R.D. 1977. Prevention of *Salmonella typhimurium* infection in poultry by pre-treatment of chickens and poults with intestinal extracts. Aust. Vet. J. 53:82-87.
41. LU J., IDRIS U., HOFACRE C., MAURER J.J., LEE M.D. AND HARMON B. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl. Environ. Microbiol. 69:6816-6824.
42. MÁTÉOVÁ S., ŠÁLY J., TUČKOVÁ M. ET AL., . 2008. Effect of probiotics, prebiotics and herb oil on performance and metabolic parameters of broiler chickens. Medycyna Weterynaryjna 64:294-297.
43. MEYER D. 2008. Prebiotic dietary fibres and the immune system. Agro-Food Ind. Hi-Tech. 19:12-15.
44. MIDILLI M., ALP M., KOCABAGLI N. Et al., . 2008. Effects of dietary probiotic and prebiotic supplementation on growth performance and serum IgG concentration of broilers. S. African J. Anim. Sci. 38:21-27.
45. MOKHTARI R., YAZDANI A.R., REZAEI M. AND GHORBANI B. 2010. The effects of different growth promoters on performance and carcass characteristics of broiler chickens. J. Anim. Vet. Adv. 9:2633-2639.
46. MOSENTHIN R. AND BAUER E. 2000. The potential use of prebiotics in pig nutrition. Asian-Austral. J. Anim. Sci. 13:315-325.
47. MURPHY M. 2004. Bacteria could treat symptoms of autism. Chem. Indust. (London):6.
48. NAKASHIMADA Y., MICHINAKA A., WATANABE K., NISHIO N. AND FUJII T. 2011. Brewer's yeast cell wall affects microbiota composition and decreases *Bacteroides fragilis* populations in an anaerobic gut intestinal model. J. Biosci. Bioeng. 111:178-184.
49. NEMCOVA R., BOMBA A., GANCARCIKOVA S. ET al., . 2007. Effects of the administration of lactobacilli, maltodextrins and fructooligosaccharides upon the adhesion of *E. coli* O8:K88 to the intestinal mucosa and organic acid levels in the gut contents of piglets. Vet. Res. Comm. 31:791-800.
50. NURMI E. AND RANTALA M. 1973. New aspects of *Salmonella* infection in broiler production. Nature 24:210-211.
51. POUILLART P.R., DEPEINT F., ABDELNOUR A. et al., . 2010. Nutrióse, a prebiotic low-digestible carbohydrate, stimulates gut mucosal immunity and prevents TNBS-induced colitis in piglets. Inflamm. Bowel Dis. 16:783-794.
52. PRICE K.L., TOTTY H.R., LEE H.B. et al., . 2010. Use of *Saccharomyces cerevisiae* fermentation product on growth performance and microbiota of weaned pigs during *Salmonella* infection. J. Anim. Sci. 88:3896-3908.
53. PROHASZKA L. AND BARON F. 1983. Antibacterial effect of volatile fatty acids on *Enterobacteriae* in the large intestine. Acta. Vet. Hung. 30:9-16.
54. RESPONDEK F., GOACHET A.G. AND JULLIAND V. 2008. Effects of dietary short-chain fructooligosaccharides on the intestinal microflora of horses subjected to a sudden change in diet. J. Anim Sci. 86:316-323.

55. RUSSELL J.B. 2002. *Rumen microbiology and its role in ruminant nutrition*. Ithaca, NY.
56. SCHIERACK P., WIELER L.H., TARAS D. et al., . 2007. *Bacillus cereus* var. *toyoi* enhanced systemic immune response in piglets. Vet. Immunol. Immunopath. 118:1-11.
57. SCHREZENMEIR J. AND DE VRESE M. 2001. Probiotics, prebiotics, and synbiotics-approaching a definition. Am. J. Clin. Nutr. 73(Suppl.):354s-361s.
58. SEIFERT S. AND WATZ B. 2007. Inulin and oligofructose: Review of experimental data on immune modulation. J. Nutr. 137:2563S-2567S.
59. SHEN J., ZHANG B., WEI H. Et al., . 2010. Assessment of the modulating effects of fructo-oligosaccharides on fecal microbiota using human flora-associated piglets. Arch. Microbiol. 192:959-968.
60. SHOAF K., MULVEY G.L., ARMSTRONG G.D. AND HUTKINS R.W. 2006. Prebiotic galactooligosaccharides reduce adherence of enteropathogenic *Escherichia coli* to tissue culture cells. Infect. Immun. 74:6920-6928.
61. SILVA V.K., DA SILVA J.D.T., GRAVENA R.A., MARQUES R.H., HADA F.H. AND DE MORAES V.M.B. 2010. Yeast extract and prebiotic in pre-initial phase diet for broiler chickens raised under different temperatures. Rev. Brasil. Zootec. 39:165-174.
62. STEER T., CARPENTER H., TUOHY K. AND GIBSON G.R. 2000. Perspectives on the role of the human gut microbiota and its modulation by pro and prebiotics. Nutr. Res. Rev. 13:229-254.
63. TORRES-RODRIGUEZ A., HIGGINS S.E., VICENTE J.L.S. et al., . 2007. Effect of lactose as a prebiotic on turkey body weight under commercial conditions. J. Appl. Poult. Res. 16:635-641.
64. TURNBAUGH P.J., HAMADY M., YATSUNENKO T. ET AL., . 2009. A core gut microbiome in obese and lean twins. Nature 457:480-484.
65. TURNBAUGH P.J., LEY R.E., MAHOWALD M.A., MAGRINI V., MARDIS E.R. AND GORDON J.I. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444:1027-131.
66. VAN LOO J. 2007. How chicory fructans contribute to zootechnical performance and well-being in livestock and companion animals. J. Nutr. 137:2594S-2597S.
67. VANDEPLAS S., DUBOIS DAUPHIN R., BECKERS Y., THONART P. AND THEWIS A. 2010. *Salmonella* in chicken: Current and developing strategies to reduce contamination at farm level. J. Food Prot. 73:774-785.
68. VOS A.P., VAN ESCH B.C., STAHL B. ET AL., . 2007. Dietary supplementation with specific oligosaccharide mixtures decreases parameters of allergic asthma in mice. Int. Immunopharmacol. 7:1582-1587.
69. WALKER W.A. AND DUFFY L.C. 1998. Diet and bacterial colonization: Role of probiotics and prebiotics. J. Nutr. Biochem. 9:668-675.
70. WALSH M.C., GARDINER G.E., HART O.M. ET al., . 2008. Predominance of a bacteriocin-producing *Lactobacillus salivarius* component of a five-strain probiotic in the porcine ileum and effects on host immune phenotype. FEMS Microbiol. Ecol. 64:317-327.
71. WELLS J.E., YEN J.T. AND MILLER D.N. 2005. Impact of dried skim milk in production diets on *Lactobacillus* and pathogenic bacterial shedding in growing-finishing swine. J. Appl. Microbiol. 99:400-407.
72. WIEMANN M. 2003. How do probiotic feed additives work? Int. Poultry Prod. 11:7-9.
73. WILLARD M.D., SIMPSON R.B., COHEN N.D. AND CLANCY J.S. 2000. Effects of dietary fructooligosaccharide on selected bacterial populations in feces of dogs. Amer. J. Vet. Res. 61:820-825.
74. WOLIN M.J. 1969. Volatile fatty acids and the inhibition of *Escherichia coli* growth by rumen fluid. Appl. Microbiol. 17:83-87.
75. XAVIER B.M. AND RUSSELL J.B. 2006. Bacterial competition between a bacteriocin-producing and a bacteriocin-negative strain of *Streptococcus bovis* in batch and continuous culture. FEMS Microbiol. Ecol. 58:317-322.
76. YASUDA K., HASHIKAWA S., SAKAMOTO H., TOMITA Y., SHIBATA S. AND FUKATA T. 2007. A new synbiotic consisting of *Lactobacillus casei* subsp. *casei* and dextran improves milk production in Holstein dairy cows. J. Vet. Med. Sci. 69:205-208.
77. ZOETENDAL E.G., VAUGHAN E.E. AND DE VOS W.M. 2006. A microbial world within us. Molec. Microbiol. 59:1639-1650.